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**SHORT-TERM METHODS FOR ESTIMATING THE CHRONIC TOXICITY OF  
EFFLUENTS AND RECEIVING WATERS TO WEST COAST MARINE AND ESTUARINE  
ORGANISMS**

(First Edition)

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## **DISCLAIMER**

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## FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluent. The National Exposure Research Laboratory-Cincinnati (NERL-Cincinnati) conducts research to:

- Develop and evaluate analytical methods to identify and measure the concentration of chemical pollutants in drinking waters, surface waters, groundwaters, wastewaters, sediments, sludges, and solid wastes.
- Investigate methods for the identification and measurement of viruses, bacteria and other microbiological organisms in aqueous samples and to determine the responses of aquatic organisms to water quality.
- Develop and operate a quality assurance program to support the achievement of data quality objectives in measurements of pollutants in drinking water, surface water, groundwater, wastewater, sediment and solid waste.
- Develop methods and models to detect and quantify responses in aquatic and terrestrial organisms exposed to environmental stressors and to correlate the exposure with effects on chemical and biological indicators.

The Federal Water Pollution Control Act Amendments of 1972 (PL 92-500), the Clean Water Act (CWA) of 1977 (PL 95-217) and the Water Quality Act of 1987 (PL 100-4) explicitly state that it is the national policy that the discharge of toxic substances in toxic amounts be prohibited. Thus, the detection of chronically toxic effluents plays an important role in identifying and controlling toxic discharges to surface waters. This manual is the first edition of the west coast marine and estuarine chronic toxicity test manual for effluents. It provides standardized methods for estimating the chronic toxicity of effluents and receiving waters to estuarine and marine organisms for use by the USEPA regional programs, the state programs, and the National Pollutant Discharge Elimination System (NPDES) permittees.

## PREFACE

This manual contains whole effluent toxicity (WET) test methods considered by USEPA's Office of Research and Development (ORD) to have the necessary characteristics for use in the NPDES program and other USEPA monitoring activities, in Pacific coastal waters, for estimating the chronic toxicity of effluents and receiving waters. All the species included in this report are currently specified in NPDES permits in one or more of the west coast states. The methods will likely be revised to some extent, especially if they are proposed in the Federal Register as 304(h) methods. Revisions would be made based upon comments received as a result of the proposed rule public comment period.

With one exception, other than changes necessary to identify the test species used in these methods and corrections of an editorial nature, the first ten sections of this document are identical to the first ten sections of the "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Estuarine and Marine Organisms, (Second Edition)." The exception occurs in chapter 7 where the use of synthetic (standard) dilution water for NPDES permit-related toxicity testing is not required. Validation and precision tests with natural seawater and HSB prepared from natural seawater (plus reagent water as necessary) have been acceptable, and synthetic waters have shown mixed results in limited testing.

The marine toxicity test procedures in this manual have been developed or refined by EPA and the states of California and Washington over a period of years. A significant number of organizations and individuals have contributed to this effort. A list of contributors is provided in the acknowledgements section. Among the major efforts that contributed critical data and critical analysis of the methods in this manual the following were vital:

- 1) The California Marine Bioassay Project (MBP). In 1984, the California State Water Resources Control Board initiated the MBP to develop sensitive methods for testing the toxicity of discharges to California marine waters. The MBP was funded wholly or in part by the USEPA using Section 205(j) grant funds. The MBP developed the tests with abalone (*Haliotis rufescens*),

topsmelt (*Atherinops affinis*), giant kelp (*Macrocystis pyrifera*), and mysid (*Holmesimysis costata*).

2) The EPA West Coast Marine Complex Effluent Program. Started in 1985, this program provided preliminary work for the topsmelt (*Atherinops affinis*), revision of methods for echinoid sperm with the purple sea urchin (*Strongylocentrotus purpuratus*) and the sand dollar (*Dendraster excentricus*), preparation of all methods into a standardized format, coordination of efforts among the various states and EPA regions 9 and 10, and development of yet unadopted test methods with the mysid (*Mysidopsis intii*) and the kelp (*Laminaria saccharina*).

3) The Protocol Review Committee (PRC) for the Triennial Review of the Marine Toxicity Test Protocols for the California Ocean Plan. In 1994 this committee reviewed a number of proposed test methods for inclusion in the California Ocean Plan. The methods included in this report are those recommended by the Protocol Review Committee. The *Mysidopsis intii* method developed by EPA was excluded from the recommended procedures because it was considered redundant with the *Holmesimysis costata* procedure. It was excluded from this report because its inclusion was also considered unnecessary by EPA region 10. The *Laminaria saccharina* test was excluded from the California recommendations because it was considered redundant with the *Macrocystis pyrifera* test. It was excluded from this report because the results from the West Coast Marine Species Chronic Protocol Variability Study indicated that more experience with the method was needed to produce acceptable precision.

4) West Coast Marine Species Chronic Protocol Variability Study. This study was a result of a 1991 settlement agreement among the Northwest Pulp and Paper Association, the Washington Dept. of Ecology, Puget Sound Water Quality Authority, and Tulalip Tribes of Washington. The year-long study in 1993-94 included monthly or quarterly interlaboratory toxicity test evaluation of tests with bivalve molluscs (*Crassostrea gigas*) and mussels (*Mytilus* sp.), echinoid sperm tests with purple sea urchins (*S. purpuratus*) and sand dollar (*D. excentricus*), sexual reproduction of kelp (*L. saccharina*), and the topsmelt (*A. affinis*).

Following review and recommendations by the PRC to the State of California for use of the procedures in this report, EPA (OR&D

and Region 9) modified the format for all methods to provide consistency among the methods as well as consistency with existing EPA Whole Effluent Toxicity Testing Manuals.

Review of the results from tests using the methods in this report indicated that they are analogous to, and as sensitive as, the methods previously proposed for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms (U.S. EPA 1994). The primary exception is the suite of invertebrate embryo-larval tests contained in this manual. These tests have been in regulatory and monitoring use on the west coast, some for many years. They tend to be more sensitive test organisms to many chemicals and the tests are more robust statistically. They have no analog in the previous EPA methods manuals, although a similar test has been proposed by the EPA laboratory in Narragansett for use in monitoring sediment-associated contaminants with the bivalve *Mulinia lateralis*.

## ABSTRACT

This manual describes six short-term (forty minutes to seven days) estuarine and marine methods for measuring the chronic toxicity of effluents and receiving waters to eight species: the topsmelt, *Atherinops affinis*; the mysid, *Holmesimysis costata*; the sea urchin, *Strongylocentrotus purpuratus* and sand dollar *Dendraster excentricus*; the red abalone *Haliotis rufescens*; the bivalves *Crassostrea gigas* and mussel *Mytilus spp.* and the giant kelp, *Macrocystis pyrifera*. The methods include single and multiple concentration static renewal and static nonrenewal toxicity tests for effluents and receiving waters. Also included are guidelines on laboratory safety, quality assurance, facilities, and equipment and supplies; dilution water; effluent and receiving water sample collection, preservation, shipping, and holding; test conditions; toxicity test data analysis; report preparation; and organism culturing, holding, and handling. Examples of computer input and output for Dunnett's Procedure, Probit Analysis, Trimmed Spearman-Kärber Method, and the Linear Interpolation Method are provided in the Appendices.

## ACKNOWLEDGEMENTS

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Section 1 through 10 of this manual are only slightly modified from the same sections in the EPA Manual, "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms" (Second Edition) and are essentially the work of Klemm, D.J., G.E. Morrison, T.J. Norberg-King and W.H. Peltier. The numerous contributors to their manual are acknowledged therein.

Four of the seven methods in this manual were adapted from methods developed by the California State Water Resources Control Board's Marine Bioassay Project. These methods for red abalone, topsmelt, mysids, and kelp were prepared by the following staff from the University of California, Santa Cruz:

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The sea urchin and sand dollar sperm tests and the bivalve mollusc embryo/larval development tests are ERL-N contributions 287 and 288, respectively, and were prepared by EPA staff:

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